forces and flexibility only in key regions of a model, capturing extensive conformational rearrangements at low cost. I use ICFF to turn low-resolution density maps, crystallographic structures, and biochemical information into the largest-scale all-atoms trajectory of ribosomal translocation modeled to date. ICFF is three orders of magnitude faster than the most comparable existing method. The results suggest an intriguing possible mechanism of translocation.

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Single Molecule Tracking of Elongation Factor Tu (EF-Tu) in Live E. Coli

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Translation requires efficient peptidyl transfer to support the cellular needs. Of the factors that facilitate the peptide elongation, elongation factor Tu (EF-Tu) is crucial in helping the correct aminoacyl-tRNA to be incorporated into the protein. It also acts as an energy carrier to move aminoacyl-tRNA near to P site peptides, forming a new peptide bond. Here, we investigated diffusive motions of translationally fused EF-Tu (TufA and TufB) with mEos2 via single particle tracking photoactivation localization microscopy (spt-PALM) on live E. coli cells. The mean diffusion coefficient of EF-Tu tracked at 100 Hz was 0.15 um^2/s. The spatial distribution of EF-Tu is reminiscent of that of translating ribosomes and its diffusion coefficient is five times faster than 70S diffusion (0.03 um^2/s). However, this is still much slower compared to the expected diffusion of free EF-Tu-size proteins such as GFP (5 um^2/s). This suggests that EF-Tu spends most of the time on ribosome or/and on a large complex. We will further investigate the cause of slow diffusive motion via antibiotic treatments and mutations on translation machinery.

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Study of Polyribosomal Effects on Frameshifting

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Frameshifting (FS) is a mechanism that enables the cell to use the same gene transcript to translate into different protein products. Frameshifting has been found mostly in prokaryotes and viruses. In prokaryotes, the mRNA for frameshifting usually contains three elements: a secondary structure (e.g., a hairpin), a slippery sequence and a Shine-Dalgarno (SD) sequence. When the ribosome is decoding the slippery sequence and stalled by the downstream structure and the upstream SD sequence, translocation of the ribosome may be affected and it will move backward by one base, resulting in −1 frameshifting.

In our research, we use the FS motif from the E. coli dnaX gene as the model. We include three tandem repeats of the FS motif. It has been shown that the FS efficiency is about 80%. However, our experiments show that the efficiency was only about 30% at the first FS site, and about 80% at second and third FS sites. We speculate that polyribosomes may play a role for the results; when one ribosome still occupies the hairpin sequence and prevents its refolding, the following ribosome will encounter no structures and thus the FS efficiency is decreased. With a mutated sequence that effectively reduces the density of ribosomes on the mRNA, the FS efficiency of the first site was recovered back to 80%. Other experiments that change the extent of polyribosomes are ongoing to further explore the effects of polyribosomes on frameshifting.

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Characterization of Ribosomal Bypassing through a 50-Nt mRNA Untranslated Gap

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The prokaryotic ribosome bypasses a 50-nt untranslated region in the gene60 mRNA of bacteriophage T4, with a ~50% bypassing efficiency. Multiple cis-acting signals are required for efficient ribosome bypassing. How the conformations of the ribosome and the mRNA change in response to these elements remains elusive.

We have developed an in vitro translation assay to assess the effects of these elements. Using complementary DNA-oligos, we can mask the mRNA sequences and the structural elements, which allow us to determine whether the ribosome is actually ''scanning'' through the region after take-off. We can also tune the concentrations of each translation factor, including Release Factor 1 (RF1) that recognizes the UAG stop codon at the 5'-junction of the gap. Interestingly, the ribosome bypassing efficiency did not exceed ~50% even in the complete absence of RF1. Additionally, the drop in the protein yield under such condition may reflect the inability for the ribosome to be liberated by RF1 and consequently a lower ribosome turn-over rate. These observations indicate the existence of another drop-off pathway independent of the RF-mediated termination.

The bypassing efficiency seems to be affected by ribosome loading rate, and that multiple unexpected ribosome drop-off products were found when bypassing was blocked by the DNA-oligos. The possibility arises that both the upstream ribosomes and the stable mRNA hairpin could push the ribosome at the take-off site to slide through the untranslated gap after the tRNA:mRNA interaction is weakened by the nascent peptide signal. Thus, the directionality of sliding could be provided without additional energy expenditure during bypassing.

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Rapid and Stable Transfer RNA Translocation through the Ribosome Ensured by Specific Contact Mechanisms

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We combined high-resolution crystal structures with cryo-EM maps of 13 intermediate states of ribosomal factorless spontaneous retro-translocation to obtain structures of the 70S ribosome in each of the 13 states in atomic detail. We then performed 100 ns all-atom, explicit-solvent molecular dynamics simulations for each of these states. Intrinsic rates for key ribosomal motions between the states were estimated from the short time fluctuations of the L1-stalk, the tRNAs and intersubunit rotations. The rates revealed rapid, submicrosecond motions of the L1-stalk and the 30S subunit. Surprisingly, it is tRNA motions, rather than large-scale intersubunit rotations, that are rate limiting for most transitions. The interaction free energy profile of the L1-stalk with the tRNA obtained from additional umbrella sampling simulations of the L1-stalk/tRNA interactions revealed an active role of the L1-stalk in pulling the tRNA from the P to the E site. Further, by detailed analysis of the frequency of contacts between 50S ribosomal proteins L5 and L16 and the tRNAs, we identified specific residues which guide the tRNAs between the binding sites. A sequence analysis of the L1, L5 and L16 proteins revealed that the conservation score for contacting residues is significantly above average. Different types of contacts characterize the interplay of these proteins with the tRNAs and involve 1) sliding of L5 residues along the tRNA elbow 2) stepping of the tRNA between L16 contact patches 3) final pulling of the tRNA by the L1-stalk. These contact mechanisms can explain how both rapid translocation and a stable tRNA binding affinity can be achieved despite large-scale displacements.

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Translational Traits of Non-Small-Cell Lung Carcinoma with Drug Resistances by Dual mRNA and Protein Quantification at the Single Cell Level

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Translation in eukaryotic cells is a fundamental biological process that has been investigated widely for understanding gene expression. To comprehensively understand gene expression, single cell level observation of both transcript and protein is desirable for translational analysis. The simultaneous observation of both transcript and protein, however, remains challenging due to the requirements of a highly sensitive and multi-modal platform. Here, we report a robust microdevice enabling both transcript and protein quantification from a single cell with a large number of cells per assay. Using 25600 microwells, we quarantined single cells for observing protein expression through immunostaining and sequentially mRNA expression via single cell reverse transcription polymerase chain reaction. Based on this correlation between transcript and protein, 2-dimensional cytometric representation from single cells is possible. The relationship between transcript and proteome of three NSCLC cell lines (HCC827, H1650 and H1975) with different levels of drug resistances to epidermal growth factor tyrosine kinase inhibitors clearly indicates unique translational traits of each cell line. Our data demonstrated that the most drug resistant cell line, H1975, showed least translational efficiency in cMET whereas the least drug resistant cell line, HCC827, showed most translational efficiency. Cytometric platforms, like the one introduced here, may ultimately allow researchers to quantify the evolution of cancerous tissues enabling detailed monitoring of tumor progression.